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Most human breast cancer cells are derived from epithelial cells. Previously studies indicated that about 20% of breast cancer patients carrying tumor cells with overexpression of Her-2/Neu. Herceptin, an antagonist antibody against Her-2/Neu, has been used in clinic for the treatment of breast cancers overexpressing Her-2/Neu. We found that EGFR and Her-2/Neu are highly overexpressed in tumor cells in approximately 30% and 23% of human breast cancer tumor samples, respectively. Interestingly, Overexpression of EGFR and Overexpression of Her-2/Neu do not overlap in most of breast cancer tumor samples. These results suggested that patients with breast cancers should receive different treatments based on their gene expression profiles. For example, breast cancer patients carrying tumors with overexpression of Her-2/Neu should be treated with Her-2/Neu antagonists such as Herceptin, while patients carrying tumors with overexpression of EGFR should be treated with EGFR antagonists.

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## INTRODUCTION

Most human breast cancer cells are derived from epithelial cells. Previously studies indicated that about 20% of breast cancer patients carrying tumor cells with overexpression of Her-2/Neu. Herceptin, an antagonist antibody against Her-2/Neu, has been used in clinic for the treatment of breast cancers overexpressing Her-2/Neu. However, most of breast cancers with normal levels of Her-2/Neu do not response well with Herceptin. The mechanisms for the development of these breast cancers need to be elucidated in order to design alternative approaches.

## BODY

This proposed research was based on our previous studies on potential mechanism for the development of nasopharyngeal carcinoma (NPC). Most of NPC are associated with Epstein-Barr Virus (EBV). We hypothesized that the EBV-encoded Latent Membrane Protein 1 (LMP-1)-mediated up-regulation of both the pro-proliferative molecule, EGFR, and the anti-apoptotic protein, Bcl-XL, are key events in the formation of EBV-associated malignancies. Accordingly, we obtained EBV-positive biopsy specimens of NPC and stained them for LMP-1, EGFR, and Bcl-XL. In all cases, we found that both EGFR and Bcl-XL were strikingly up-regulated in the tumor, but not in the surrounding normal tissues (unpublished data). These results confirm those reported by Sheen et. al. in which they found that roughly 70% of NPC tumors stained positively for both LMP-1 and EGFR. We have suggested that EGFR as a potential therapeutic target to treat human patients carrying NPC.

The new discovery of EBV association with breast cancer led us to the hypothesis that LMP-1-mediated up-regulation of EGFR and Bcl-XL may also contribute to the formation of malignant breast cancer. Recent studies detecting the viral protein EBNA-1 have shown a highly variable incidence (0 to 50%) of EBV in breast cancer biopsies. To determine the levels of EGFR and the pro-survival Bcl-XL protein, we will perform immunohistochemical stainings on LMP-1 expressing tumors, using normal breast tissue biopsies as controls. If, as expected, EGFR and Bcl-XL are overexpressed in breast tumors, we will gain information as to the etiology of these tumors and will also be presented with a new therapeutic target for the treatment of breast cancer. Xenograph models would then be used to test the efficacy of EGFR as a therapeutic target for breast cancer. It is our vision that this work will lead to clinical trials using EGFR as a target for novel therapy for breast cancer.

## KEY RESEARCH ACCOMPLISHMENTS

We have obtained over 30 human breast cancer samples from UCLA tissue bank. We have used the immunohistochemical staining method to detect the expression of determine their expression of EGFR, Her-2/Neu and Bcl-x in tumor cells and their surrounding normal tissues

with the corresponding antibodies. We have converted the expression levels of EGFR, Her-2/Neu and Bcl-x into numeric scales from 0, 1+, 2+, 3+ representing no staining, weak staining, strong staining, and highly overexpressed.

## REPORTABLE OUTCOMES

| Specimen # | EGFR | | HER-2NEU | | BCL-X | |
|---|---|---|---|---|---|---|
| | Non-tumor | Tumor | Non-tumor | Tumor | Non-tumor | Tumor |
| S01-3086 | | 3+ | | 0 | | 3+ |
| S01-15814 | 1+ | 1+ | 0 | 0 | 1+ | 3+ |
| S01-11043 | 0 | 1+ | 0 | 0 | 2+ | 2+ |
| S01-14826 | | 0 | | 1 | | 2+ |
| S01-8859 | 0 | 0 | 0 | 2+ | 2+ | 3+ |
| S01-5983 | 0 | 1+ | 0 | 2+ | 2+ | 3+ |
| S01-16364 | 0 | 2+ | | 0 | 0 | 3+ |
| S01-8752 | | 2+ | | 1 | | 3+ |
| S01-7079 | | 1+ | | 0 | | 3+ |
| S01-14316 | | 0 | | 1 | | 3+ |
| S01-7814 | 2+ | 0 | | 0 | 3+ | 3+ |
| S01-2427 | 1+ | 0 | | 1 | 3+ | 3+ |
| S01-15729 | | 1+ | | 0 | | 2+ |
| S01-1230 | | 1+ | | 1 | | 3+ |
| S00-20922 | 1+ | 2+ | | 2+ | 3+ | 3+ |
| S00-18452 | | 1+ | | 1 | | 3+ |
| S00-16106 | 0 | 0 | | 2+ | 2+ | 3+ |
| S00-8906 | | 0 | | 1 | | 3+ |
| S00-509 | | 3+ | | 1 | | 2+ |
| S00-482 | | 2+ | | 1 | | 3+ |
| S99-22690 | | 0 | | 0 | | 3+ |
| S99-20319 | | 2+ | | 0 | | 3+ |
| S99-18130 | 0 | | | 0 | 2+ | 3+ |
| S99-17231 | 0 | | | | | 2+ |
| S01-10274 | 0 | 0 | | 2+ | 2+ | 3+ |
| S01-6851 | 0 | 1+ | | 0 | 2+ | 3+ |
| S00-12837 | 0 | 2+ | | 2+ | 3+ | 3+ |
| S01-12592 | 1+ | 0 | | 2+ | 2+ | 3+ |
| S01-5741 | 1+ | 2+ | | 1 | 2+ | 3+ |
| S01-6322 | | 0 | | 0 | | 3+ |

## CONCLUSIONS

1. We found that EGFR is highly overexpressed (equal or greater than 2+) in tumor cells in approximately 30% of human breast cancer tumor samples.

2. Her-2/Neu is expressed higher (2+) in tumor cells than their surrounding normal cells in approximately 23% of human breast cancer tumor samples.

3. Overexpression of EGFR and Overexpression of Her-2/Neu do not overlap in most of breast cancer tumor samples. These results suggested that patients with breast cancers should receive different treatments based on their gene expression profiles. For example, breast cancer patients carrying tumors with overexpression of Her-2/Neu should be treated with Her-2/Neu antagonists such as Herceptin, while patients carrying tumors with overexpression of EGFR should be treated with EGFR antagonists. Our studies also raised several interesting questions for future studies: 1). Is overexpression of one members of the EGFR family sufficient to lead to the development of malignancies? 2). Is overexpression of EGFR or Her-2/Neu in human breast cancers related to the geographic locations or ethic background of patients? 3). What are the molecular mechanisms of EGFR and Her-2/Neu overespression? Is EGFR overexpression related to EBV infection?

4. Bcl-x is expressed in both normal breast tissues and tumor cells, but its levels in breast cancer cells are higher in many cases than normal breast tissues.

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## APPENDICES